# AN ELECTRON MICROSCOPE STUDY OF VERO CELLS INFECTED WITH HOMOGENEOUS AND HETEROGENEOUS VIRUS OF VEE

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ELECTRON MICROSCOPE STUDY OF VERO CELLS INFECTED WITH GENETICALLY HOMOGENEOUS AND HETEROGENEOUS VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

B. V. Gushchin, Ya. Ya. Tsilinsky, L. S. Shushkov, D. K. Lvov, S. M. Klimenko

The electron microscope examination of Vero cells infected with a mixture of clone 6 and clone No 8 demonstrated that at 17 and 23 hours after inoculation simultaneously with formation of mononucleoid virions comprising the main part of virus particles, multivirus particles containing several separate nucleoids are produced. At later intervals, 29 and 41 hours after infection, giant virus particles are formed containing some material of density to that of nucleoids. It is suggested to name the former type of particles "polynucleoid virions" and the latter "giant virus particles". No formation of polynucleoid forms and giant virus particles were observed in the cells infected with individual clones. These facts suggest that infection of the cells with genetically heterogeneous VEE virus is necessary condition for production of polynucleoid virions and giant virus particles.

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# AN ELECTRON-MICROSCOPE STUDY OF VERO CELLS INFECTED WITH HOMOGENEOUS AND HETEROGENEOUS VIRUS OF VENEZUELAN EQUINE ENCEPHALOMYELITIS

[Paper by B. V. Gushchin, Ya. Ya. Tsilinskiy, L. S. Shushkov, D. K. L'vov and S. M. Klimenko, Institute of Virology D. I. Ivanovskiy, USSR Academy of Medical Sciences, Moscow; published in Voprosy Virusologii (Problems of Virology), 18 April 1973; received by the editors 1 August 1972]

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The authors previously reported [1-3, 5, 6] that in suspensions obtained following infection of cells with the virus of a wild strain of Venezuelan equine encephalomyelitis (VEE), or with a mixture of clones, two types of virions are found to be present: standard (mononucleoid) virions measuring 60-90 nm. and giant virions (di- and polynucleoid) measuring 120-300 nm. which have not yet been observed in suspensions prepared from single clones.

The present report includes new data on the types of giant virions formed in Vero cells infected with genetically nonhomogeneous VEE virus (a mixture of clones No. 6 and No. 8).

## Material and Methods

The Vero cells were grown on Medium 199, with addition of 10% normal ox serum. To infect the cells, clones No. 6 and No. 8 of the VEE virus were used, data on which were previously published [1, 2, 5, 6].

Multiplicity of innoculation during infection with single clones was 1 toxic unit per 1,000 cells. When mixed infection was used, each clone was introduced in the amount of 1 unit per 2,000 cells. The cells were removed from the glass mechanically 17, 23, 29 and 41 hours following infection. They were then washed three times in 0.15 M solution of phosphate buffer (pH 7.3) and centrifuged at 700 rpm for 5 minutes. The resulting sediments were fixed in a 2.5% solution of glutaraldehyde, 1% solution of $SOO_4$, dehydrated, and ...[text unclear]...in an epone-araldite mixture. The sections were prepared with a "Huxley" ultramicrotome (Cambridge, England), contrasted with 1% solution of uranyl acetate on methanol [4] and lead citrate [7], and, finally, studied under the JEM-100-B electron microscope.

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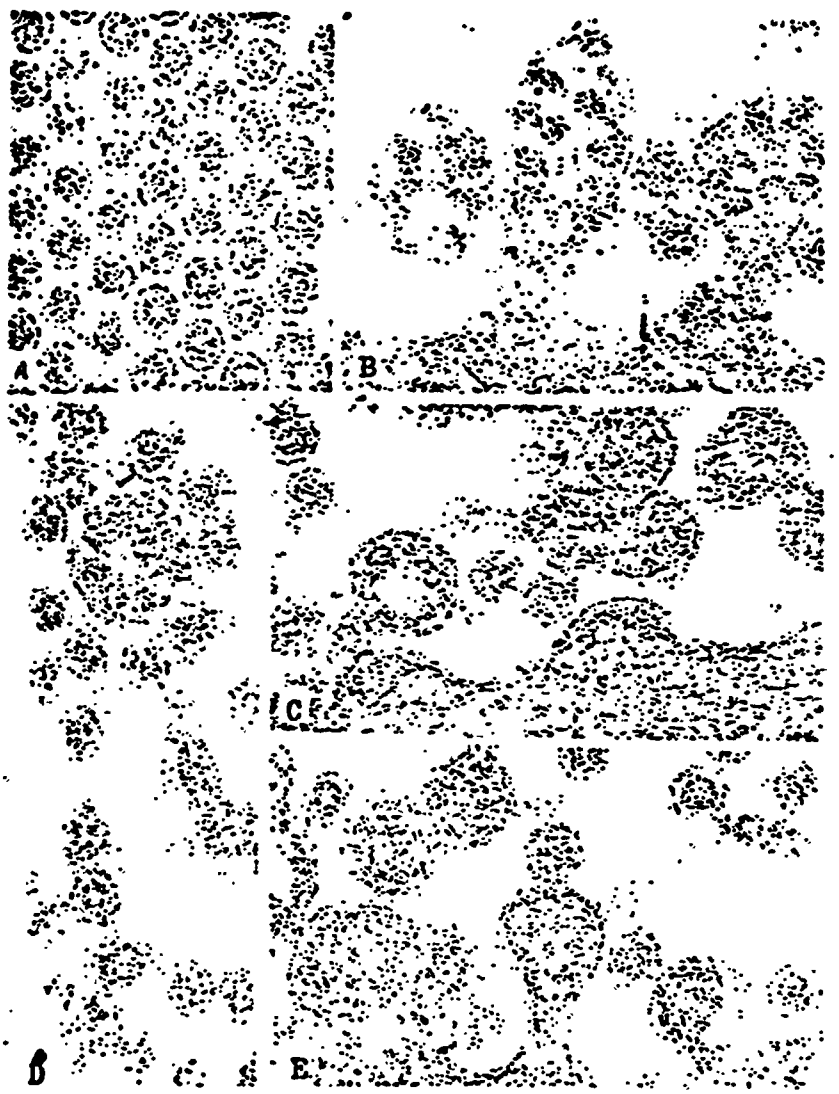


Virions Observed in Vero Cells Infected with Single Clones and with a Mixture of Clones: A - Collection of mononucleoid virions in intercellular space following infection; B - Portion of a cell surface 28(?) hours following infection with a mixture of clones; C - Polynucleoid virion in intercellular space. Magnification, 200,000. D and E - Part of a cell surface ...[text unclear]... following infection with a mixture of clones. Initial stages in the formation of giant cells. Magnification, 200,000.

## Results

As revealed by the electron-microscope study of ultrafine sections of Vero cells infected with single clones, 23 hours following infection with clone No. 6, and 17 and 23 hours following infection with clone No. 8, various stages in the formation of mononucleoid virus particles are observable on the cell surfaces. At these same times there were observed inclusions within within the cell cytoplasm, these consisting of optically dense spherical structures which evidently consisted of accumulations of the precursors of the nucleoids. Twenty-nine and 41 hours following infection (in the case of both clone No. 6 and clone No. 8), the greater part of the cells had been destroyed, and the spaces between their remains had been filled by mononucleoid virions (Illustration, A).

In the ultrafine sections of Vero cells infected with a mixture of clones Nos. 6 and 8, 17 and 23 hours following innoculation, in addition to the formation of mononucleoid virions (comprising the basic mass of viral particles, it was possible to observe various stages in the formation of giant virions with distinctly defined nucleoids (Illustration, B and C). It should be noted tat a majority of the giant cells observed in these media exhibited a whole set of morphological signs characteristic of VEE virus particles [3]. In the later stages (29 and 41 hours following infection), giant virions with distinctly defined nucleoides were only rarely observed. Instead, on the cell surfaces and also in the intercellular spaces were found giant particles containing either several indistinctly different nucleoids, or else material equivalent in density to nucleoid substances (Illustration, D and E). In all cases, giant forms of this type possessed an external membrane.

## Discussion

In 1971, on the basis of results obtained with use of negative contrast in the study of suspensions of single clones, clone mixtures, and a wild strain of VEE virus [1, 2, 5, 6], the authors were able to suggest that one necessary factor in the formation of giant virions is the infection of cells with genetically nonhomogeneous VEE virus. The data given in the present study support the direct conclusion that in Vero cells infected with clones Nos. 6 and 8 of VEE virus there is formation both of mononucleoid and giant virions; whereas, in the case of infection with single clones, it is only mononucleoid particles which are formed.

It should be noted that the use of our method based on ultrafine sections made it possible to discern certain details in the fine structure of giant virions of the VEE virus. As the data given here substantiate, giant virions can be classed in two basic types—particles containing two or more nucleoids within the same membrane, and particles containing material of the same density as nucleoids. In this connection, it would be beneficial to refer to particles of the first type as "polynucleoid virions", and those of the second as "giant virus particles".